M·H

TENT COOPERATION TRE /

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 11 February 2000 (11.02.00)	
International application No. PCT/GB99/01848	***Applicant's or agent's file reference*** P21993/GST/RMC
International filing date (day/month/year) 10 June 1999 (10.06.99)	**Priority date** (day/month/year) 10 June 1998 (10.06.98)
Applicant NELSON, John et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

10 January 2000 (10.01.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO ***34, chemin des Colombettes*** **1211 Geneva 20, Switzerland**	Authorized officer Jean-Marc Vivet
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P21993/GST/RMC		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/01848	10/06/1999	10/06/1998
International Patent Classification (IPC) or national classification and IPC C07K14/00		
Applicant THE QUEEN'S UNIVERSITY OF BELFAST et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10/01/2000	19.09.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Roscoe, R Telephone No. +49 89 2399 2554

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/01848

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-15 as originally filed

Claims, No.:

1-10 as originally filed

Drawings, sheets:

1/14-14/14 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4, 8
	No:	Claims	1-3, 5-7, 9, 10
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-10
Industrial applicability (IA)	Yes:	Claims	1-8
	No:	Claims	9, 10

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Citations

The documents mentioned in the present International Preliminary Examination Report are numbered as in the search report, i.e. D1 corresponds to the first document of the search report etc.

V. **Reasoned statement on Novelty, Inventive Step and Industrial Applicability**

- **Novelty (Art.33(2) PCT)**

D1 discloses synthetic peptides for use in tumor therapy. The peptides comprise 17aa signal sequence "ERQIKIWFQNRRMKWKK" linked to functional sequences RRYRRDFAEM(FP1) or AAPAPGIFS(FP2) and variants thereof. In addition to the signal sequences, FP1 functional domain adds arginine (R) residues to the peptide. Further , it is impossible for the authorized authority to determine the extent of the hydrophobic core of the above signal peptide and thus which parts of the 17aa sequence can be considered as "additional". Certainly, it is assumed that the terminal lysine residues do not form part of the core. D1 anticipates claims 1-3, 5-7, 9 and 10.

D2 discloses peptides comprising Karposi fibroblast H region linked to peptides having lysine residues (see Fig.1). These peptides are cited against claims 1-5, 7, 9 and 10. The same applies to the peptides in Fig.1 of D4 and SP1173 in Fig.1 of D6.

- **Inventive Step (Art.33(3) PCT)**

D2 states on p.239, col.2 "This idea is..." that preferably positive residues are required for membrane transport in the N-terminal region. However, it is not suggested to add positive residues to already membrane transportable molecules or to add them to the core region.

Applicant argues that inventive activity is based on the fact that the positively charged amino acids have the effect of rendering the hydrophobic cores of signal peptide molecules more water-soluble without compromising their ability to

translocate across cellular membranes. This was not disclosed or suggested in the cited prior art. This solubility problem does however not exist when the entire signal peptide is used. The claims are however drafted so that the signal peptide component is defined as being also possibly the entire signal peptide and it is also not specifically stated that the core must be modified. In this case, applicant solves no problem and inventive activity cannot be acknowledged. Hence, the present claims are not considered inventive.

- **Industrial Applicability (Art.33(4) PCT)**

For the assessment of the present claims 9 and 10 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Claims 9 and 10 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

VIII. **Certain observations**

- **Clarity (Art.6 PCT)**

Claim 1 is unclear. A non-specific peptide is modified in a non-specific manner. The analog refered to could be one lacking a positive aa. Addition of such an aa to such an analog could result in the original protein again.

Substantially similar in claim 2 is unclear - basis to render clear e.g. p.3 description.

Claim 4 - modified analog problem.

- **Support in Description (Art.6 PCT)**

Applicant only experiments with modified K-FGF with a modified treble-lysine. He does not show that the effect of increased solubility / membrane permeability is achieved in any other signal peptide or with any other number, particularly lower, of positively charged amino acids. Hence, the claims in their present breadth are considered unsupported.

A·P

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
P21993/GST/RMC		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/ 01848	10/06/1999	10/06/1998
Applicant		
THE QUEEN'S UNIVERSITY OF BELFAST et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   CELL-PERMEABLE PEPTIDE

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

[redacted]national Application No
PCT/GB 99/01848

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/50 C12N15/87 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 04006 A (HERRMANN FRIEDHELM ;BRACH MARION (DE); KIEHNTOPF MICHAEL (DE)) 6 February 1997 (1997-02-06) the whole document ---	1-3,5-7, 9,10
A	DU C ET AL., : "Conformational and topological requirements of cell-permeable peptide function" JOURNAL OF PEPTIDE RESEARCH, vol. 51 (3), March 1998 (1998-03), page 235-243 XP000856550 the whole document ---	1-10

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 December 1999	23/12/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mateo Rosell, A.M.

3

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	ALLINQUANT B ET AL., : "Downregulation of amyloid precursor protein inhibits neurite outgrowth in vitro" THE JOURNAL OF CELL BIOLOGY, vol. 128, no. 5, March 1995 (1995-03), pages 919-927, XP000856389 cited in the application abstract page 920, left-hand column, paragraph 3 page 923, right-hand column, paragraph 2 -page 925, right-hand column, last paragraph	1,9,10
A	LIN Y-Z ET AL., : "Inhibition of nuclear translocation of transcription factor NF-kappaB by a synthetic peptide containing a cell membrane-permeable motif and nuclear localization sequence" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 24, 1995, pages 14255-14258, XP002050723 the whole document	1,2,9
A	FULLER-PACE F ET AL., : "Cell transformation by kappaFGF requires secretion but not glycosylation" J. CELL. BIOL. , vol. 115, no. 2, 1991, pages 547-555, XP000856406 page 547 -page 548	4
A	ROJAS M. ET AL., : "Controlling epidermal growth factor (EGF)-stimulated Ras activation in intact cells by a cell-permeable peptide mimicking phosphorylated EGF receptor" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 44, 1996, page 27456-27461 XP002124151 the whole document	1,2,9

INTERNATIONAL SEARCH REPORT

[redacted]tion on patent family members

[redacted]ational Application No
PCT/GB 99/01848

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9704006 A	06-02-1997	DE 19526174 A	23-01-1997

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

09719245

(43) International Publication Date
16 December 1999 (16.12.1999)

PCT

(10) International Publication Number
WO 99/064449 A3

(51) International Patent Classification[6]: C07K 14/50, C12N 15/87, A61K 31/70

(21) International Application Number: PCT/GB99/01848

(22) International Filing Date: 10 June 1999 (10.06.1999)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9812376.3 10 June 1998 (10.06.1998) GB
9814888.5 10 July 1998 (10.07.1998) GB

(71) Applicant *(for all designated States except US)*: **THE QUEEN'S UNIVERSITY OF BELFAST** [GB/GB]; 8 Malone Road, Belfast BT9 5BN (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **NELSON, John** [GB/GB]; 59 Ashley Avenue, Belfast BT9 7BU (GB). **HARRIOTT, Patrick** [GB/GB]; 12 William Alex Park, Finaghy, Belfast BT10 0LW (GB). **WALLACE, Andrew** [GB/GB]; 49 Flat, 2 University Street, Belfast BT7 1FY (GB).

(74) Agent: **MURGITROYD & COMPANY**; 373 Scotland Street, Glasgow G5 8QA (GB).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
24 October 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 99/064449 A3

(54) Title: CELL-PERMEABLE PEPTIDE

(57) Abstract: The present invention relates to a new method of delivery of molecules into a cell through the use of a modified signal peptide to which a peptide nucleic acid is linked. The signal peptide will comprise at least one positively charged amino acid residue, or functional equivalent thereof. The addition of such positively charged residues can serve as a linker group for the attachment of peptide nucleic acids to the signal peptide thus increasing the number of peptide nucleic acid sequences delivered by the signal peptide and therefore its functional efficiency. Extension of the signal peptide at the C or N terminus through the addition of a single or multiple charged residue or analogues thereof will modify and improve signal peptide delivery function by increasing the solubility and cell permeability characteristics of the signal peptide.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/01848

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/50 C12N15/87 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 04006 A (HERRMANN FRIEDHELM ;BRACH MARION (DE); KIEHNTOPF MICHAEL (DE)) 6 February 1997 (1997-02-06) the whole document	1-3,5-7, 9,10
A	DU C ET AL., : "Conformational and topological requirements of cell-permeable peptide function" JOURNAL OF PEPTIDE RESEARCH, vol. 51 (3), March 1998 (1998-03), page 235-243 XP000856550 the whole document	1-10

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 December 1999	23/12/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Mateo Rosell, A.M.

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ALLINQUANT B ET AL., : "Downregulation of amyloid precursor protein inhibits neurite outgrowth in vitro" THE JOURNAL OF CELL BIOLOGY, vol. 128, no. 5, March 1995 (1995-03), pages 919-927, XP000856389 cited in the application abstract page 920, left-hand column, paragraph 3 page 923, right-hand column, paragraph 2 -page 925, right-hand column, last paragraph	1,9,10
A	LIN Y-Z ET AL., : "Inhibition of nuclear translocation of transcription factor NF-kappaB by a synthetic peptide containing a cell membrane-permeable motif and nuclear localization sequence" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 24, 1995, pages 14255-14258, XP002050723 the whole document	1,2,9
A	FULLER-PACE F ET AL., : "Cell transformation by kappaFGF requires secretion but not glycosylation" J. CELL. BIOL. , vol. 115, no. 2, 1991, pages 547-555, XP000856406 page 547 -page 548	4
A	ROJAS M. ET AL., : "Controlling epidermal growth factor (EGF)-stimulated Ras activation in intact cells by a cell-permeable peptide mimicking phosphorylated EGF receptor" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 44, 1996, page 27456-27461 XP002124151 the whole document	1,2,9

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/01848

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9704006 A	06-02-1997	DE 19526174 A	23-01-1997

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 14/00

A2

(11) International Publication Number: WO 99/64449

(43) International Publication Date: 16 December 1999 (16.12.99)

(21) International Application Number: PCT/GB99/01848

(22) International Filing Date: 10 June 1999 (10.06.99)

(30) Priority Data:

9812376.3	10 June 1998 (10.06.98)	GB
9814888.5	10 July 1998 (10.07.98)	GB

(71) Applicant *(for all designated States except US)*: THE QUEEN'S UNIVERSITY OF BELFAST [GB/GB]; 8 Malone Road, Belfast BT9 5BN (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: NELSON, John [GB/GB]; 59 Ashley Avenue, Belfast BT9 7BU (GB). HARRIOTT, Patrick [GB/GB]; 12 William Alex Park, Finaghy, Belfast BT10 0LW (GB). WALLACE, Andrew [GB/GB]; 49 Flat, 2 University Street, Belfast BT7 1FY (GB).

(74) Agent: MURGITROYD & COMPANY; 373 Scotland Street, Glasgow G5 8QA (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: PEPTIDE

(57) Abstract

The present invention relates to a new method of delivery of molecules into a cell through the use of a modified signal peptide to which a peptide nucleic acid is linked. The signal peptide will comprise at least one positively charged amino acid residue, or functional equivalent thereof. The addition of such positively charged residues can serve as a linker group for the attachment of peptide nucleic acids to the signal peptide thus increasing the number of peptide nucleic acid sequences delivered by the signal peptide and therefore its functional efficiency. Extension of the signal peptide at the C or N terminus through the addition of a single or multiple charged residue or analogues thereof will modify and improve signal peptide delivery function by increasing the solubility and cell permeability characteristics of the signal peptide.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

"Peptide"

The present invention relates to the delivery of molecules into a cell and the use of modified signal peptides.

Specifically, a modified analogue of the signal peptide sequence from Karposi syndrome fibroblast growth factor (kFGF) is used as a cell-permeant vehicle for the intracellular delivery of covalently linked anti-sense peptide nucleic acid sequences (PNAs).

PNAs have potential uses as antisense molecules for the control of gene expression. Since they are capable of binding tightly to DNA and RNA targets thus preventing DNA transcription to RNA and RNA translation to protein. These molecules thus have two potential uses of commercial importance:

1. As research reagents where scientists use antisense strategies to ablate selected genes in order to understand their function.

2. As pharmaceutical compounds for companies seeking to develop nucleic acid-based therapies.

Conventional anti-sense oligonucleotide *in vivo* delivery is highly inefficient, even if long-lasting, less polar phosphorothioates are used.

This invention covers the use of cell-permeant peptide delivery systems based on the hydrophobic core sequences of any signal peptide sequence. A signal peptide is a short-lived *N*-terminal sequence found only on nascent proteins which are synthesised in the endoplasmic reticulum. Signal peptides consist of three domains:

(a) *N*-terminus of 1-5 amino acids, often positively charged;

(b) A hydrophobic core or central region (7-16 amino acids) which is essential for translocation across the endoplasmic reticulum membrane; and

(c) A more polar C-terminal domain (3-7 amino acids) which is important for specifying the cleavage site.

Synthetic peptides consisting of only the hydrophobic cores are typically insoluble in water. Taking the signal peptide sequence of Karposi syndrome-derived FGF as an example, we have modified these insoluble sequences by the addition of positively charged amino acids (for example lysines), which have the effect of rendering them water soluble without compromising their ability to translocate across cellular membranes. The ability to add amino groups in this way allows extra cargo sequences to be conjugated to these amino groups.

It is an object of the present invention to provide a cell permeable peptide delivery system based on a

signal peptide sequence for the intracellular delivery of peptide nucleic acid sequence.

According to the present invention there is provided a cell permeable peptide comprising at least the hydrophobic core of a signal peptide or an analogue thereof wherein the peptide is modified by the addition of at least one positively charged amino acids or positively charged analogues thereof.

The signal peptide may be a natural or synthetic signal peptide or a peptide which is substantially similar thereto.

A peptide which is substantially similar to a signal peptide is at least 60% homologous thereto.

At least one positively charged amino acid is chosen from lysine and/or arginine and/or any positively charged analogues thereof.

In one particular embodiment the cell permeable peptide is a modified analogue of Karposi syndrome fibroblast growth factor (kFGF).

The positively charged amino acid consists of one or more lysine residues.

The invention further provides the use of cell permeable peptides as described herein for intracellular delivery of a molecule.

Preferably, one or more lysine residues will be attached to the C terminal of the signal sequence peptide or signal sequence peptide analogue.

This positively charged lysine allows the linkage of a peptide nucleic acid, thus facilitating in vivo delivery of the said peptide nucleic acid.

The invention also provides a cell permeable peptide which contains multiple positively charged amino acids or positively charged analogues thereof wherein a peptide nucleic acid may be conjugated to each positively charged residue and wherein the peptide nucleic acids conjugated by such a means are identical or different.

The invention also provides a cell permeable peptide which comprises at least one positively charged amino acid residue or functionally equivalent positively charged analogue thereof conjugated or conjugatable to a lysine tree, to which multiple peptide nucleic acids may be joined for transport and presentation.

The linked peptide nucleic acid sequence may be antisense.

Preferably, the peptide nucleic acid sequence will be covalently linked.

The present invention thus allows the use of cell permeable peptides as described herein to deliver peptide nucleic acids to in-vivo targets.

Use of conventional oligonucleotides is being reduced due to the development of PNAs (Neilsen, et al., 1991), which are much more stable, being resistant to enzymic degradation (Jordan, et al., 1997). PNAs replace the phosphodiester backbone of nucleic acid with repeating N-(2-aminoethyl)glycine units to which natural

nucleobases are attached through methylenecarbonyl linkers. Although more stable, PNAs suffer from similar accessibility problems as phosphorothioates do, and passive diffusion of unmodified PNA across lipid membranes is not efficient (Wittung, P., et al., 1995).

A small number of native peptide sequences can translocate across membranes of living cells in an energy-independent and receptor-independent manner. These peptides have been used to import active cargo into the cell. For example a peptide from the homeodomain of *Antennapedia* has been successfully used to import both peptidal inhibitors of protein kinase C (Theodore, et al., 1995) and conventional anti-sense oligonucleotides (Allinquant, et al., 1995).

The present invention provides use of cell permeable peptide import (CPPI) to deliver peptide nucleic acids (PNAs).

The present invention provides use of the signal peptide sequence from Karposi syndrome fibroblast growth factor (kFGF) for delivery of antisense peptide nucleic acid sequences (PNAs).

The invention provides use of a peptide as defined herein together with lysine residues for multiple presentation of peptide nucleic acids.

The invention further provides use of peptides as defined herein together with lysine residues in the simultaneous presentation of different peptides nucleic acids.

The present invention combines the two above technologies to use CPPI to deliver PNAs to *in vivo*

targets.

The invention described herein has the following advantages:

- The modified signal peptides described in this invention can be used for the delivery of any cell-impermeant substance into cells.

- The signal peptides described in this invention can be used to improve the delivery of substances of low permeability into cells.

- The delivery of substances to particular cellular sub-compartments can be achieved and improved by incorporating appropriate targeting peptide sequences or other modifications to the signal peptides. Effects are only due to the 'cargo' substance that they carry. For example, addition of a myristoyl moiety to the peptide would ensure that it was preferentially retained at the plasma membrane.

- The signal peptide delivery system has commercial value in therapeutic drug-delivery systems including, but not restricted to, gene therapy, cancer therapy and anti-infectious agent therapy.

- This system also has commercial value as a tool for biochemical and molecular biological research.

- The modified signal peptides described in this invention do not, themselves, exhibit any biological effects nor do they affect cell viability. Effects are only due to the 'cargo' substance that they carry.

This invention will be exemplified in the following non-limiting examples with reference to the accompanying figures wherein:-

Figure 1 illustrates carboxyfluorescein labelled kFGF signal peptide-Lys.Lys.Lys - fluoresence calibration curve.

Figure 2 illustrates carboxfluorescein labelled cell permeant peptide incorporation by whole human endothelial cells.

Figure 3 depicts incorporation of carboxyfluorescein labelled signal peptide-Lys.Lys.Lys by cell.

Figure 4 illustrates subcellular distribution of labelled signal peptide in cells.

Figure 5 depicts incorporation of labelled kFGF peptide into human dermal endothelial cells.

Figure 6a sets out the signal peptide sequence and modifications.

Figure 6b illustrates simultaneous presentation of 3 PNAs directed to different sites on a target RNA.

Figure 6c illustrates multiple presentation of the single PNA species.

Table 1 describes carboxyfluorescein derivatised cell permeant peptides.

Table 2a sets out uptake of cell permeant peptides by cells.

Table 2b sets out cellular uptake of permeant peptides by BHK cells.

Table 3 sets out results of washing labelled antennapedia cells.

Table 4 sets out washing results for labelled signal peptide-KKK and cells.

EXAMPLE 1

This is an example of the intracellular delivery of a low molecular weight compound (carboxyfluorescein) which is normally cell impermeant.

In order to determine the best delivery system, a comparison of the ability of four different cell permeant peptides (Table 1) to accumulate in whole cells was undertaken. The four people peptides were synthesised to contain carboxyfluoresein as a reporter group (Table 1), allowing intracellular accumulation to be monitored by fluorescence. Whole cells were exposed to 50 μM solutions of each peptide for 24 hours (37°C) and accumulation was measured using a fluorometer. The results of this are shown in Tables 2A and 2B.

The results shown in the whole column of Table 2A were provided by cell suspensions being exposed to 50μM peptide each, for 24 hours at 37°C. Incubations contained 3.28 x 106 cells in 1 ml. Subcellular fractionation was then carried out. Fluorescence measured with excitation λ = 471 nm, emission λ = 521 nm. RFU valves were converted to nMoles per 10^6 cells.

The raw relative fluorescent units (RFU) values were converted to nMoles per 10^6 cells using a calibration

curve constructed for each peptide. An example of a fluorescence calibration curve of fluorescein labelled kFGF is shown in Figure 1.

The kFGF-KKK sequence (see Figure 3) shows similar high rates of cytosolic and nuclear incorporation compared with the antennapedia peptide (Table 2A). The PKC and substance P peptides show much lower incorporation Table 2A & 2B). Incorporation of the kFGF-KKK sequence is saturable, as can be seen from the data presented on Figure 2 and time-dependent as shown in Figure 3.

Table 2A shows that antennapedia is lost during subcellular fractionation. Unlike the antennapedia peptide, carboxyfluorescein-kFGF signal peptide-KKK is not loosely attached to the cell surface as shown in Tables 3 and 4. Unlike the antennapedia peptide, carboxyfluorescein-kFGF signal peptide-KKK does not remain membrane-bound as shown by the data presented in Figure 4.

It should be noted from Figure 4 that all cells treated with carboxyfluorescein - labelled kFGF signal peptide Lysine-Lysine-Lysine have nuclear and cytoplasmic incorporation. Unlike antennapedia, very little remains stuck in the cell membrane.

EXAMPLE 2 - Anti-sense agents for gene ablation

Conventional oligonucleotide sequences or those in which the phosphodiester bonds are replaced with nuclease-resistant bonds (such as the phosphothiorates and the like) may be conjugated to the kFGF-derived delivery system for intracellular delivery and subsequent specific blocking of gene translation or Rnase-targeted destruction of the mRNA in question.

Alternatively peptide nucleic acid sequences may be used, as in example 1.

Although the "cargo" to be delivered intracellularly is referred to in the text and represented in the accompanying figures as a Peptide Nucleic Acid (PNA), it should not be limited to such cargo type as the various configurations of CPPI described in this Patent could also be used to carry peptide sequences or oligonucleotide sequences (either native sequences or modified sequences, such as phosphothiorates).

It has been demonstrated that addition of a peptide nucleic acid sequence does not impede incorporation of the carboxyfluorescein-kFGF signal peptide-{PNA}-KKK. The confocal micrograph shown in Figure 5 illustrates this.

EXAMPLE 3

Nuclear localisation signal (NLS) sequences such as are found on transcription factors like NF-kappaB may be conjugated to the kFGF-derived delivery system, as in Example 1. Intracellular delivery of NLS peptide sequences would act as 'bait' to selectively block the translocation of the selected transcription factor, thus preventing its action. In this way, genes under the control of the transcription factor could be identified on the basis of down regulated expression.

EXAMPLE 4

Signal transduction motifs such as phosphotyrosine-containing peptide sequences (pYP's) act as docking sites for a large number of proteins. Such signalling proteins contain domains that recognise (contextually)

the phosphotyrosine residues and bind to them in a specific manner. pYP's are recognised by SH-2(Src-homology-2) domains and PTB (phosphotyrosine binding domains). Specificity is provided by short amino acid sequences *N*-and/or *C*-terminal of the phosphotyrosine. Such peptide motifs could be conjugated to the kFGF peptide-derived delivery system as in Example 1, and could be used to intracellularly deliver pYP's which would act as bait, thus allowing signal pathways to be 'interrogated'.

The signal sequence of kFGF was modified to contain three lysines at the C-terminal of the hydrophobic signal sequence. This procedure is illustrated in Figure 6A. In this Figure 6A (I) shows the signal peptide with an attached reporter group. Figure 6A Part II illustrates the addition of the tri-lysine extension to the C-terminal of the signal peptide sequence, thus providing three positive charges which aid solubility and cell permeability. In Figure 6A Part IIIb, the peptide nucleic acid forms part of the linear primary amino acid sequence, with Part IV illustrating a tri-lysine C-terminal extension to the peptide nucleic acid sequence providing 3 positive charges and aiding solubility and cell permeability.

Part V of Figure 6A further shows a tri-lysyl extension at the N-terminal of the signal peptide which provides 3 positive charges aiding solubility and cell permeability. The addition of the tri-lysyl extension proximal to the carboxyfluorescein reporter group enhances its fluorescence. In Vb of Figure 6A, the peptide nucleic acid sequence initially forms part of the linear primary amino acid sequence at the N-terminal of the original peptide, before a tri-lysyl extension is added to the N-terminal of the peptide

nucleic acid extension.

It should be noted that although the above examples specifically use the amino acid lysine for the addition of positive charge, molecules containing similar properties such as arginine or analogues thereof, of either of these molecules could also be used.

This peptide, therefore, can accommodate three PNAs, each bonded to a lysine epsilon amino group. This can be extended using the Multiple Antigen Presentation (MAP) technology to present eight (or more) PNA's on one kFGF signal sequence. A 'lysine tree' constructed in this way accommodates eight copies of the same PNA, thus increasing the effective concentration delivered by each CPPI.

An example of the addition of such a lysine tree is shown in Figure 6C Parts I-IV. In Part I a single lysine molecule added to the C-terminal of the kFGF signal peptide sequence allows the multiple PNA lysine tree to be added to the e-amino group of the lysine side chain.

Alternatively, Part II of Figure 6C a lysine molecule added to the N-terminal of the kFGF signal peptide sequence allows the multiple PNA lysine tree to be added to the e-amino group of the lysine side chain.

Part III of Figure 6C further shows that when a C-terminal tri-lysine extension is added to the signal peptide with N-terminal associated multiple PNA lysine tree, the 3 positive charges aid solubility and cell permeability of the molecule.

Part IV of Figure 6C add a tri-lysyl extension at the

N-terminal of the signal peptide which is attached to the lysine group added to allow attachment of the multiple PNA lysine tree as originally illustrated in Figure 6C Part II. The addition of the 3 positively charged molecules at this terminal of the molecule, proximal to the carboxyfluorescein reporter group enhances its fluorescence.

Alternatively a carrier can be constructed containing three (or more) different PNAs directed towards different sites on the same target mRNA. This strategy has been termed 'molecular triangulation' (Branch, A.D., 1998).

Figure 6B illustrates this process of 'molecular triangulation'. Figure 6B Part I shows the signal peptide with a C-terminal tri-lysyl extension which allows three different PNA sequences to be conjugated to the epsilon-amino groups of the three lysines.

Figure 6B Part III shows the addition of a further three lysines to the molecule of Part I, which adds three positive charges, which aid solubility and cell permeability. Figure 6B Part III shows the addition of the tri-lysyl extension to the N-terminal of the molecule of Part I. Again the 3 positive charges aid the solubility and cell permeability of the molecule, which their proximal location to the carboxyfluorescein reporter group enhances its fluorescence.

Figure 6B, Part IV, illustrates an N-terminal tri-lysyl extension added to the kFGF signal peptide sequence, which subsequently allows three different PNA sequences to be conjugated to the epsilon-amino groups of the lysines.

Further, this molecule has 3 lysines added at the C-terminal to add positive charge which aid solubility and cell permeability. Figure 6B Part V shows the signal peptide again with the three peptide nucleic acid associated tri-lysine extension at the N-terminal, but with the addition of the further 3 lysine groups also being made to the N-terminal where they will have the effect of aiding solubility and cell permeability, which also enhance the fluorescence of the carboxyfluorescein reporter group due to their proximity.

Further to the sequences illustrated in Figures 6A and 6C additional tri-lysine extensions at either end of the molecule, appears to aid solubility and cell permeability to allow PNA sequences to be transported. Therefore in addition to using lysine residues to attach to PNA sequences, additional tri-lysine extension is recommended. Examples of presentation peptide using the additional try-lysine is demonstrated in Figures 6B (II-IV), Figures 6C (III-IV) and Figures 6A (IV, IVb, V, Vb).

Lysine extensions comprising more or less than three lysine residues may also be useful to provide additional solubility and cell permeability.

The lysine extension may be provided next to a carboxyfluorescein reporter group to enhance its fluorescence.

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Branch, A.D. (1998) A good antisense molecule is hard to find. TIBS, **23**: 45-50.

Jordan, S., Schwemler, C., Kosch, W., Kretschner, A., Schwenner, E., Stropp, U. and Mielke, B. (1997) Synthesis of new building blocks for peptide nucleic acids containing monomers with variations in the backbone. *Bioorg. Med. Chem. Lett.*, 7: 681-686.

Neilsen, P.E., Egholm, M., Berg, R.H. and Buchardt, O. (1991) Sequence-selective recognition of RNA by strand displacement with a thymine-substituted polyamide. *Science*, 254: 1497-1500.

Theodore, L. Derossi, D., Chassang, G., Llirbat, B., Kubes, M., Jordan, P., Chneiweiss, H., Godement, P. and Prochiantz, A. (1995) Intraneuronal delivery of protein kinase C pseudosubstrate leads to growth cone collapse. J. Neurosci., **15**: 7158-7167.

Wittung, P., Kajanus, J., Edwards, K., Haaima, G., Nielson, P., Norden, B. and Malmstrom, B.G. Phospholipid membrane-permeability of peptide nucleic-acid (1995) *FEBS Lett.*, **375**: 317-320.

CLAIMS

1 A cell permeable peptide comprising at least the hydrophobic core of a signal peptide or an analogue thereof wherein the peptide is modified by at least the addition of at least one positively charged amino acid or positively charged analogue thereof.

2 A cell permeable peptide as claimed in claim 1 wherein the signal peptide is a natural or synthetic signal peptide or a peptide which is substantially similar thereto.

3 A cell permeable peptide as claimed in claim 1 and 2 wherein at least one positively charged amino acid is chosen from lysine and/or arginine and/or any positively charged analogue thereof.

4 A cell permeable peptide as claimed in any preceding claim wherein the cell permeable peptide is a modified analogue of Karposi syndrome fibroblast growth factor (kFGF).

5 A cell permeable peptide as claimed in any preceding claim where in the positively charged amino acid consists of one or more lysine residues.

6 A cell permeable peptide as claimed in claim 5 wherein one or more lysine residues are attached to the C-terminal of the signal sequence peptide or signal sequence peptide analogue.

7 A cell permeable peptide as claimed in any of claims 1 to 6 which contains multiple positively

charged amino acids or positively charged analogues thereof, wherein a peptide nucleic acid may be conjugated to each positively charged residue and wherein the peptide nucleic acids conjugated by such means are identical or different.

8 A cell permeable peptide as claimed in any of claims 1 to 6 which comprises at least one positively charged amino acid residue or functionally equivalent positively charged analogue thereof, conjugated or conjugatable to a lysine tree, to which multiple peptide nucleic acids may be joined for transport and presentation of multiple peptide nucleic acids.

9 Use of cell permeable peptides claimed in any of the preceding claims for intracellular delivery of a molecule.

10 Use of a cell permeable peptide as claimed in any of claims 1 to 8 to deliver peptide nucleic acids to *in-vivo* targets.

Figure 1

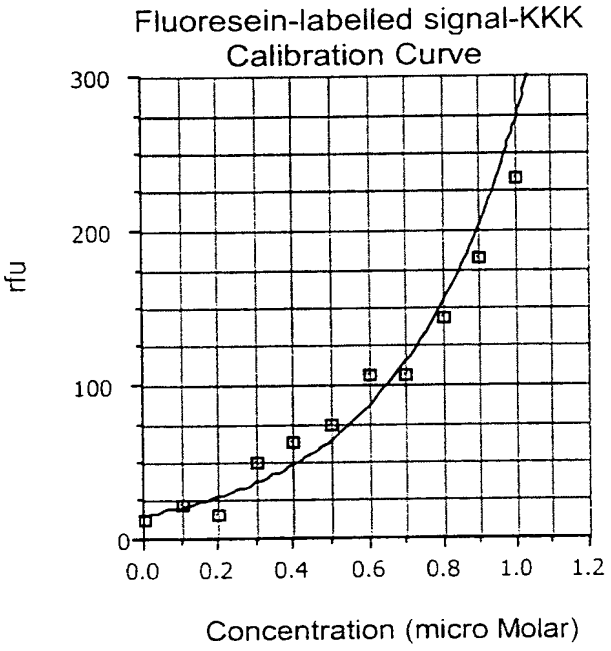

Fluoresein-labelled signal-KKK
Calibration Curve
300
200
100
0
rfu
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Concentration (micro Molar)

Figure 2

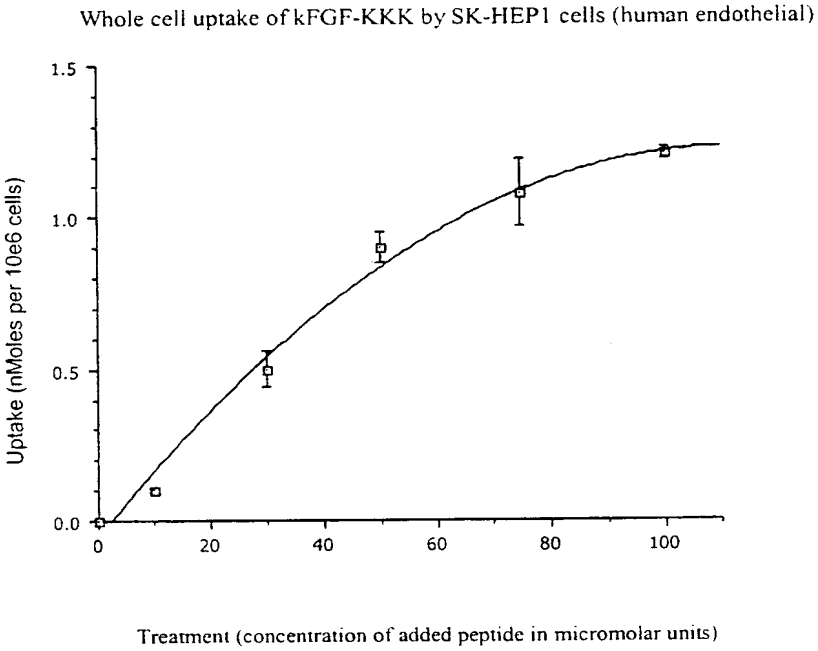

Whole cell uptake of kFGF-KKK by SK-HEP1 cells (human endothelial)
Uptake (nMoles per 10e6 cells)
1.5
1.0
0.5
0.0
0
20
40
60
80
100
Treatment (concentration of added peptide in micromolar units)

Figure 3

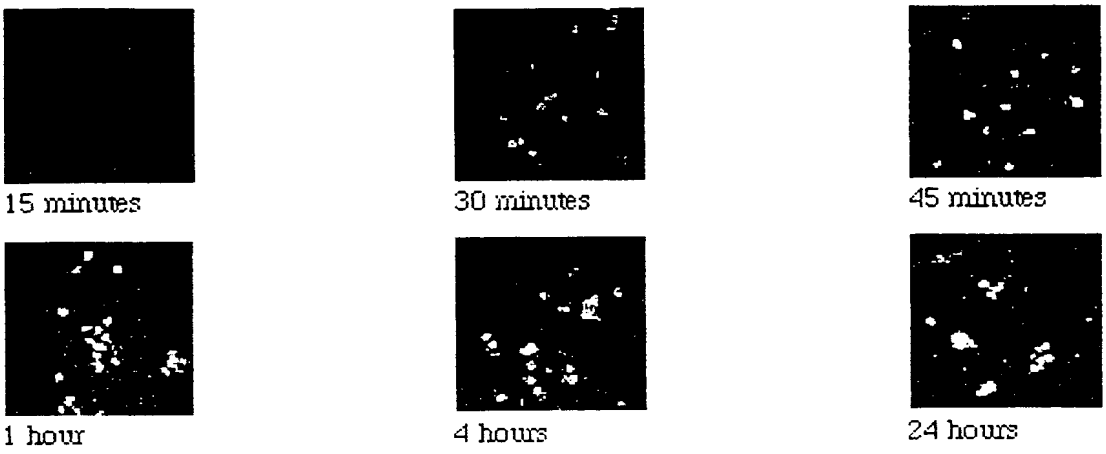


Figure 4

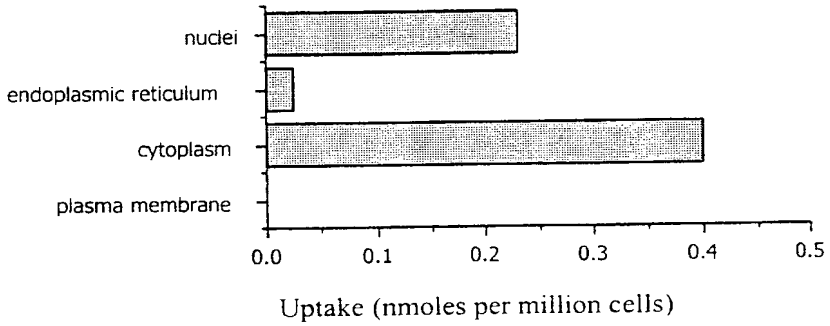

nuclei
endoplasmic reticulum
cytoplasm
plasma membrane
0.0
0.1
0.2
0.3
0.4
0.5
Uptake (nmoles per million cells)

Figure 5

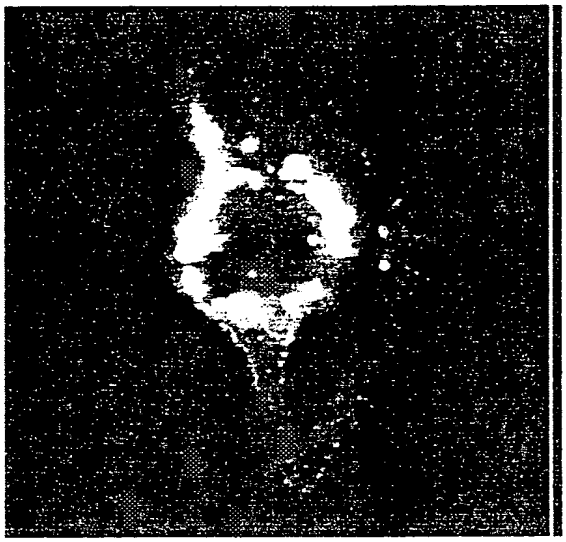


Figure 6A

A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P *6A(I).*

CarboxyFluor -A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P *6A(II)*

CarboxyFluor -A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P. K.K.K *6A(III)*

CarboxyFluor -A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P--[PNA SEQUENCE] *6A(IIIb)*

CarboxyFluor -A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P--[PNA SEQUENCE -]-K.K.K *6A(IV)*

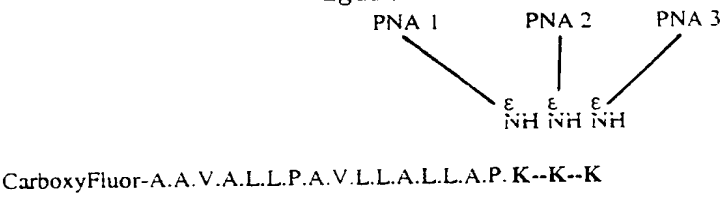
CarboxyFluor -A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P. K.K.K -[PNA SEQUENCE] *6A(IVb)*

CarboxyFluor -.K.K.K ---A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P--[PNA SEQUENCE] *6A(V)*

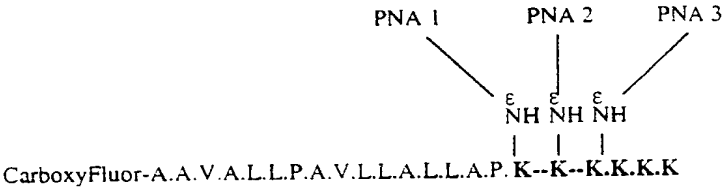
CarboxyFluor -.K.K.K -[PNA SEQUENCE -]-A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P *6A(Vb)*

Figure 6B

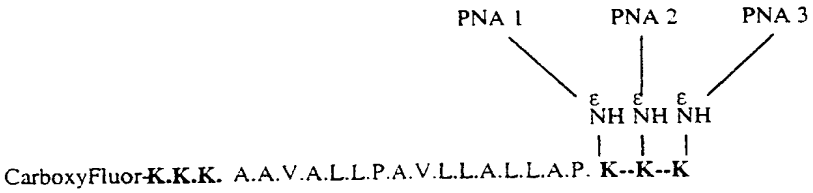
6B(I)



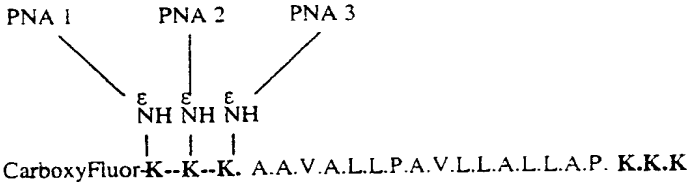
6B(II)



6B(III)



6B(IV)



6B(V)

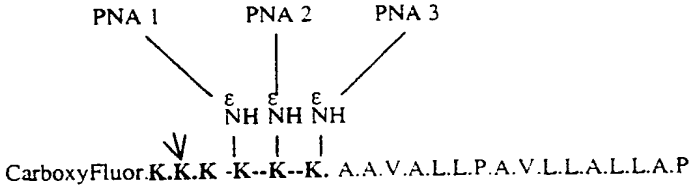
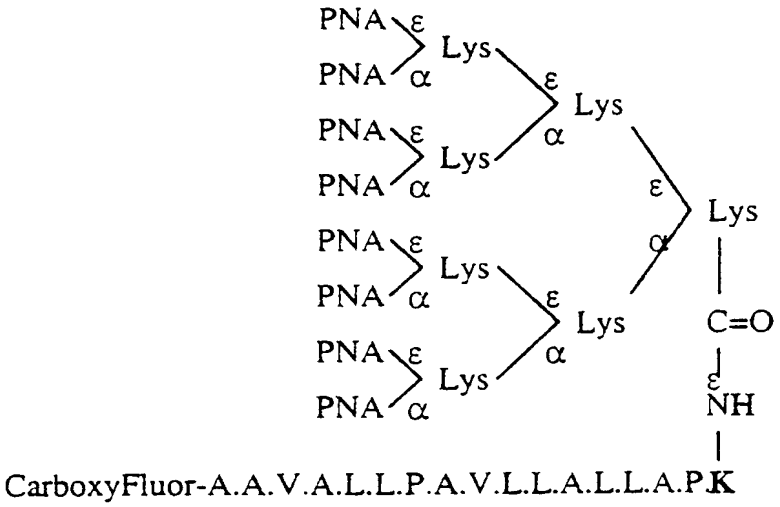
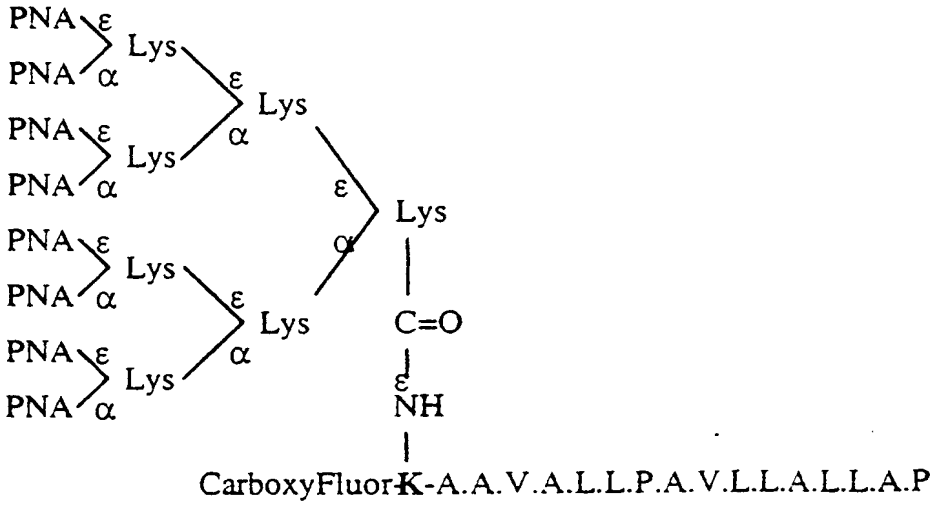


FIGURE 6C

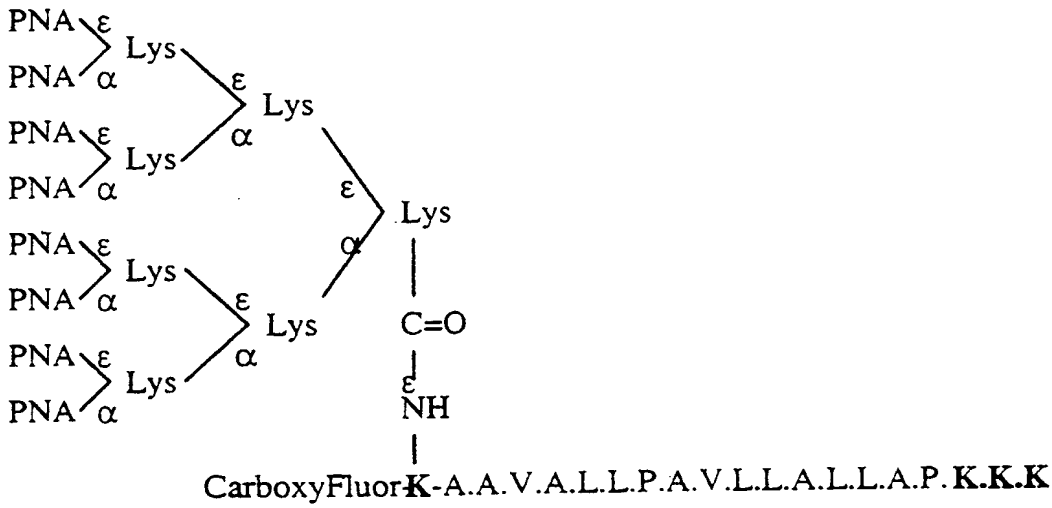
6C(I)



6C(II)



6C(III)


PNA
PNA
PNA
PNA
PNA
PNA
PNA
PNA
ε
α
Lys
Lys
Lys
Lys
Lys
Lys
Lys
C=O
NH
CarboxyFluor-K-A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P.K.K.K

6C(IV)

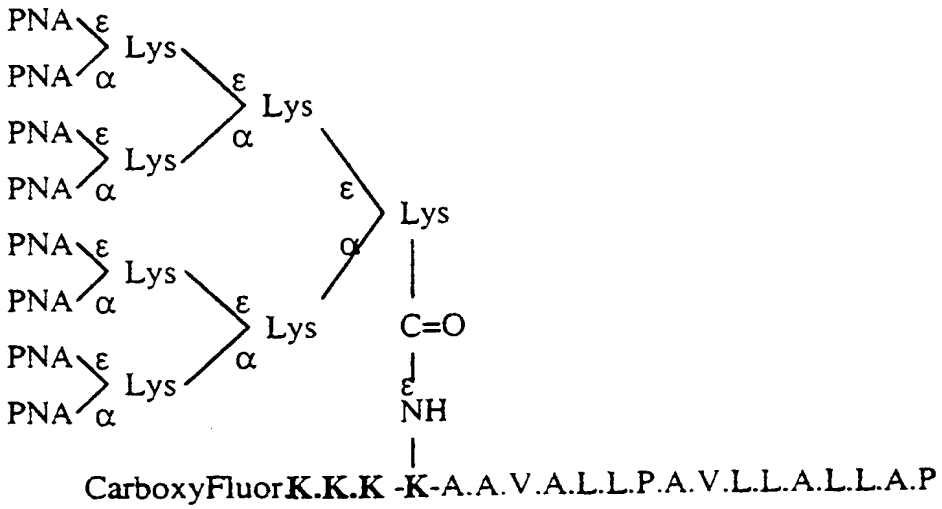

PNA
PNA
PNA
PNA
PNA
PNA
PNA
PNA
ε
α
Lys
Lys
Lys
Lys
Lys
Lys
Lys
C=O
NH
CarboxyFluorK.K.K -K-A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P

Table 1

Carboxyfluorescein-derivatised Cell Permeant Peptides*																			
kFGF signal sequence	**cFl**	A	A	V	A	L	L	P	A	V	L	L	A	L	L	A	P	**K**	**K**	**K**
PKC Pseudo - substrate	**cFl**	R	F	A	R	K	G	A	L	R	Q	K	N	V	H	E	V	K	N	
Substance P	**cFl**	R	P	R	P	Q	Q	F	**Ø**	G	L	M								
Antennapedia	**cFl**	R	Q	I	K	I	W	F	Q	N	R	R	M	K	W	K	K			

*Modifications of original sequence marked in bold (ø = ornithine, cFl = carboxyfluorescein).

Table 2A

	*WHOLE CELL nmoles per 10^6 cells	CYTOSOL nmoles per 10^6 cells	NUCLEI nmoles per 10^6 cells
FGF-KKK	0.79	0.37	0.35
KKK-FGF-KKK	0.24	0.046	0.15
Substance P	0.03	0.005	0.015
PKC pseudo - substrate	0.034	0.015	0.007
Antennapedia	1.22	0.34	0.35

*Cell suspensions were exposed to 50 μM peptide each, for 24 hours, at 37°C, =471nm, emission λ = 521nm. RFU values were converted to nMoles per 10^6 cells

Table 2B

CPPI sequence tested	Amount in nuclei (nmoles per 10^6 cells)	Amount in cytosol (nmoles per 10^6 cells)	Cytosolic concentration (μM)
kFGF signal peptide	0.035	0.0567	13.5
SubstanceP analogue	0.0005	0.0018	0.42
PKC pseudosubstrate	0.0005	0.00156	0.37

Table 3

Treatment	rfu
1st PBS wash -	114
2nd PBS	57.34
3rd	21.08
4th PBS/acid wash	15.36

Table 4

Incorporation Treatment	incorporation (nmoles per 106 cells
PBS wash (after 15min exposure)	0.64
Acid Wash (15min)	0.525
PBS wash (after 24hour exposure)	0.75
Acid wash (after 24hour exposure)	0.53